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<b>(54) Title:</b> SUBSTITUTED BENZHYDRYLAMINES AS HANDLES FOR SOLID PHASE PEPTIDE SYNTHESIS  <b>(57) Abstract</b>  The present invention is directed to a novel "handle" for solid phase peptide synthesis. The handles can be converted from a stable form to a labile form, allowing for cleavage of a peptide amide from the support after synthesis under mild conditions, and after deprotection of the amino acid side chains. The handles are based on a substituted benzhydrylamine skeleton.		

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SUBSTITUTED BENZHYDRYLAMINES AS  
HANDLES FOR SOLID PHASE PEPTIDE SYNTHESIS

1. FIELD OF THE INVENTION

5       The present invention is directed to a novel  
"handle" for solid phase peptide synthesis. The  
handles can be converted from a stable form to a  
labile form, allowing for cleavage of a peptide from  
the support after synthesis under mild conditions, and  
10   after deprotection of the protecting groups from the  
amino acid side chains. The handles are based on a  
substituted benzhydrylamine skeleton.

2. BACKGROUND OF THE INVENTION

15       As used in the art of peptide synthesis, the  
handle is defined as a bifunctional spacer that serves  
to link the peptide fragment or first amino acid to  
the polymer support. Many solid phase resins and  
handles are available in the art, as is described in  
20   Fields and Noble, 1990, Int. J. Pept. Protein Res.  
35:161-214. To be useful, handles must be stable to  
the reaction conditions of peptide synthesis, but when  
the synthesis is complete, the handle needs to allow  
for cleavage of the peptide from the solid support.  
25       A few handles that can be converted from a stable  
form of handle to a labile form have been designed and  
synthesized. Unfortunately, these handles have found  
no broad application in practice. For example, the 4-  
benzylthiophenyl- and 4-benzylsulfonyl-handle (D.L.  
30   Marshall, I.E. Liener: J. Org. Chem. 35, 867 (1970))  
and sulfonamide-handle (G.W. Kenner, J.R. McDermott,  
R.C. Sheppard: Chem. Commun. 636, (1971)) have not  
found broad application. Further examples can be  
found in "The Peptides, Analysis, Synthesis, Biology,"  
35   E. Gross, J. Meienhofer, Eds., Academic Press New  
York, vol. 2. p. 88 (1980), vol. 3. p. 209 (1981).

Quite recently, the handle based on the 4-alkylthiobenzyl alcohol has been disclosed (EP 274,998 and EP 274,999; Chem. Abstr. 110, 135705 (1989)) and used in peptide synthesis. After reduction of the sulfoxide moiety, the 4-alkylthiobenzyl ester is cleaved to give a peptide with free carboxyl group.

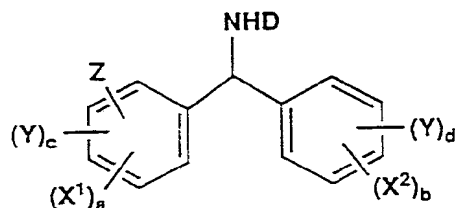
The majority of aforementioned handles have, however, certain obvious limitations concerning the amino acid residues that can be affected by the conversion step. As a rule, oxidation steps cannot be used when the peptide contains sensitive amino acids such as tryptophan, cysteine, cystine, and methionine. Another problem is possible methylation during "activation" of handle and long cleavage times which can cause the damage of peptide.

Moreover, the handles presently available are not well adapted to both Boc and Fmoc synthetic procedures.

### 3. SUMMARY OF THE INVENTION

The instant invention is directed to substituted benzhydrylamine handles for peptide synthesis. The handles are prepared and used in acid labile form or in stable form. The stable form can be converted to an acid labile form for cleavage of the peptide from the resin.

The substituted benzhydrylamine handles are of the general formula (I):



in which  $a = 1$  to  $3$ ,  $X^1$  is  $[S]R^1$  or  $X^1$  is  $Z$ , the  $X^1$  groups are in the ortho or para positions of the first benzene ring, and  $R^1$  is an alkyl group and

5        in which  $b = 0$  to  $3$ ,  $X^2$  is  $[S]R^2$ , the  $X^2$  groups are in the ortho or para positions of the second benzene ring, and  $R^2$  is an alkyl group; and

          in which  $Z$  is  $R^3$ ,  $OR^3$  or  $[S]R^3$  in any position not occupied by  $X^1$  unless  $X^1$  is  $Z$ , and  $R^3$  is an alkyl group comprising a reactive functional group for coupling to a solid phase support, and in which if  $X^1$  is  $Z$ ,  $Z$  is  $[S]R^3$  in an ortho or para position; and

          in which  $c = 0$  or  $1$  and  $d = 0$  or  $1$ ,  $Y$  is  $OR^4$ , and  $R^4$  is an alkyl group; and

15        in which  $[S]$  is  $-S-$ ,  $-SO-$  or  $-SO_2-$ ; and

          in which  $D$  is  $H$ , a protecting group or an  $N^\alpha$ -protected acyl. As used herein, "alkyl" can be a  $C_1$  to  $C_{10}$ .

          Further provided are methods for synthesis of the handles of the invention. In one embodiment, hydroxy or mercapto benzophenones are reacted with  $\omega$ -haloesters of alkanecarboxylic acids in the presence of fluoride ions. The benzophenone carbonyl is subsequently converted to an amine by routine synthetic methods, e.g., reaction with hydroxylamine to yield an oxime, followed by reduction, e.g., with zinc, to yield benzhydramine, or by reductive amination, e.g., by reaction with ammonium formate. Alternatively the benzophenone can be reduced to the alcohol and amidated with an  $N^\alpha$ -protected amino acid amide.

          The present invention offers distinct advantages over earlier methods of creating stable handles for peptide synthesis that can be treated after the synthetic reaction is complete to make the handle labile. Once the handle is converted from stable form

to labile form, the peptide can be conveniently  
cleaved from a solid phase support and used. The  
peptide is cleaved in a carboxy amide (rather than  
5 free carboxyl) form. The oxidized handles, i.e., in  
the sulfoxide or sulfone form, are extraordinarily  
stable in acidic media, e.g., trifluoroacetic acid, as  
well as conditions generally used in peptide  
synthesis. When reduced to sulfide form, the handle  
10 is acid labile. In a preferred embodiment, the  
sulfoxide or sulfone is readily reduced to sulfide, to  
convert the stable handle to a labile form.

One particular advantage of the handles of the  
invention is that they allow for deprotection of the  
15 amino acid side chains prior to cleavage. Under  
standard solid phase synthesis, the cations generated  
during deprotection and cleavage are available to  
react with the cleaved peptide. Often reaction of  
these cations with the peptide results in decreased  
20 yield and purity. According to the present invention,  
the cations generated during deprotection can be  
washed away since the deprotected peptide remains on  
the solid support. During cleavage, the cations  
remain with the solid support, away from the cleaved  
25 peptide.

Another advantage of the invention is that  
cleavage of the peptide after reduction of the handle  
can be performed under much milder conditions than are  
generally available. For example, in a standard Boc  
30 synthesis cleavage requires treatment with the  
powerful acid, usually HF. The present invention thus  
allows a Boc synthesis with the final cleavage under  
much milder conditions.

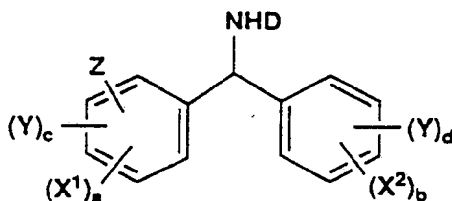
Yet a further advantage of the invention is that  
35 the handle can be used for both Fmoc and Boc synthetic  
strategies.

Yet another advantage of the handles of the invention is that cleavage yields a C-terminal peptide amide under less drastic conditions than are currently available.

#### 4. DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to handles for peptide synthesis that are stable, but that can be converted to labile form for subsequent cleavage of the peptide from the solid phase support. The stability of the handles is determined by the oxidation state of an alkyl-sulfur substituent.

The substituted benzhydrylamine handles are of the general formula (I):



in which  $a = 1$  to  $3$ ,  $X^1$  is  $[S]R^1$  or  $X^1$  is  $Z$ , the  $X^1$  groups are in the ortho or para positions of the first benzene ring, and  $R^1$  is an alkyl group; and

in which  $b = 0$  to  $3$ ,  $X^2$  is  $[S]R^2$ , the  $X^2$  groups are in the ortho or para positions of the second benzene ring, and  $R^2$  is an alkyl group; and

in which  $Z$  is  $R^3$ ,  $OR^3$  or  $[S]R^3$  in any position not occupied by  $X^1$  unless  $X^1$  is  $Z$ , and  $R^3$  is an alkyl group comprising a reactive functional group for coupling to a solid phase support, and in which if  $X^1$  is  $Z$ ,  $Z$  is  $[S]R^3$  in an ortho or para position; and

in which  $c = 0$  or  $1$  and  $d = 0$  or  $1$ ,  $Y$  is  $OR^4$ , and  $R^4$  is an alkyl group; and

in which  $[S]$  is  $-S-$ ,  $-SO-$  or  $-SO_2-$ ; and

in which D is H, a protecting group or an N<sup>α</sup>-protected acyl. As used herein, "alkyl" can be a C<sub>1</sub> to C<sub>10</sub>.

5 As used herein, the term alkyl includes but is not limited to C<sub>1</sub> to about C<sub>10</sub> alkane, alkene and alkyne (i.e., saturated and unsaturated hydrocarbons). For example, an alkyl group may be methyl, ethyl, ethenyl, propyl, propenyl, propynyl, etc. The term alkyl as  
10 used herein includes branched chain as well as linear chain groups, and cyclic alkyl.

As used herein, the term "handle" is defined as a bifunctional spacer. The handle has one functional group that binds to a solid phase support. The handle  
15 has an second functional group that can be conjugated to an amino acid or a peptide. The handle provides for cleavage of the peptide after synthesis is complete.

As used herein, the term "solid phase support" is  
20 not limited to a specific type of support. Rather a large number of supports are available and are known to one of ordinary skill in the art. Solid phase supports include silica gels, resins, derivatized plastic films, glass beads, cotton, plastic beads,  
25 alumina gels. A suitable solid phase support may be selected on the basis of desired end use and suitability for various synthetic protocols. For example, for peptide synthesis, solid phase support may refer to resins such as p-methylbenzhydrylamine  
30 (pMBHA) resin (Peptides International, Louisville, KY), polystyrene (e.g., PAM-resin obtained from Bachem Inc., Peninsula Laboratories, etc.), poly (dimethylacrylamide)-grafted styrene co-divinyl-  
benzene (e.g., POLYHIPE® resin, obtained from  
35 Aminotech, Canada), polyamide resin (obtained from Peninsula Laboratories), polystyrene resin grafted



with polyethylene glycol (TentaGel®, Rapp Polymere, Tubingen, Germany) or polydimethylacrylamide resin (obtained from Milligen/Bioscience, California).

5 In one embodiment, the solid phase support may be suitable for in vivo use, i.e., it may serve as a carrier for or support for direct applications of the peptide (e.g., TentaGel, Rapp Polymere, Tubingen, Germany). In a particular embodiment, the solid phase  
10 support may be palatable and orally consumable.

The term "peptide" is used herein in its broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs or peptidomimetics. The subunits may be linked by peptide bonds. In  
15 another embodiment, the subunit may be linked by other the bonds, e.g., ester, ether, etc. As used herein the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D and L optical isomers, and amino acid  
20 analogs and peptidomimetics. As used herein, a peptidomimetic is a molecule that exhibits properties similar to a peptide without having a peptide chemical structure. The peptides may comprise D-amino acids, a combination of D- and L-amino acids, and various  
25 "designer" amino acids (e.g.,  $\beta$ -methyl amino acids,  $\alpha$ -methyl amino acids, and N $\alpha$ -methyl amino acids, etc.) to convey special properties to peptides. Additionally, by assigning specific amino acids at specific coupling steps, peptides with  $\alpha$ -helices,  $\beta$   
30 turns,  $\beta$  sheets,  $\gamma$ -turns, and cyclic peptides can be generated.

#### 4.1. HANDLES

The benzhydrylamine handles of the invention are  
35 substituted with at least one sulfur-containing derivative selected from the group consisting of

sulfide ( $\text{SR}^1$ ), sulfoxide ( $\text{SOR}^1$ ), and sulfone ( $\text{SO}_2\text{R}^1$ ), in which  $\text{R}^1$  is an alkyl group of 1 to about 10 carbon atoms. The sulfide, sulfoxide or sulfone derivative is designated throughout this application as  $[\text{S}]\text{R}$ . The sulfur-containing group is found at the ortho position or para position on at least one benzene ring. Two sulfur-containing groups on a single benzene ring can be found at the ortho-para or both ortho positions. Three sulfur containing groups can be found at the two ortho and one para positions. The benzene rings can be symmetrically substituted with sulfur-containing groups, but need not be. Preferably both benzene rings contain a sulfur-containing group at the para or ortho position.

The handle further comprises a linker, Z, for attachment to a solid phase support, i.e., resin, for solid phase peptide synthesis using the handle. In one embodiment, the linker can be a sulfur-containing group ( $[\text{S}]\text{R}^3$ ) located in the ortho or para position of one benzene ring. In another embodiment, the linker can be an alkyl group ( $\text{R}^3$ ), alkoxy ( $\text{OR}^3$ ) group, or sulfur containing group ( $[\text{S}]\text{R}^3$ ) located at the ortho, para or meta position, with the proviso that if Z contains O or S and is in the meta position, it cannot be considered to influence the stability properties of the handle. The alkyl group on Z,  $\text{R}^3$ , has from 1 to about 10 carbon atoms, and comprises a functional group, for example, carboxylic acid, for attachment to the resin.

In a further embodiment, the handle comprises an alkoxy group ( $\text{OR}^4$ ), Y, in the ortho or para positions on one or both benzene rings not occupied by a sulfur-containing group X. No group Y is found on a ring that has Z in the ortho or para position when Z is  $\text{OR}^3$  (alkoxy). The alkoxy groups can be but need not be

symmetrically arranged on the two benzene rings. If an alkoxy group (Y) is present, the number of Y groups on a single benzene ring is less than or equal to the number of X groups on the ring; the total number of Y is less than or equal to the total number of X groups on the benzhydryl amine, and is preferably less. The alkyl portion of Y has 1 to about 10 carbon atoms.

Although the present invention is not limited to any particular mechanism, it is believed that the properties of the handle are determined by the electron donating or electron withdrawing characteristics of substituents in the ortho or para positions of the benzene ring or rings. Thus when electron donating groups, such as sulfide or alkoxy groups, are in the para or ortho positions, acidolysis of the N-C bond readily occurs because electrons can be donated to stabilize the resulting benzhydryl cation. Conversely, when an electron withdrawing group such as sulfoxide or sulfone is present in the para or ortho positions, acidolysis cannot occur. Since electron donation occurs less readily from the meta position, substituents in the meta position are expected to have orders of magnitude less effect than substituents in the para or ortho positions.

Substitution with both sulfoxide or sulfone substituents and alkoxy substituents results in a balance of electron withdrawing and electron donating groups. However, when the number of electron withdrawing oxidized sulfur-containing groups is at least equal to and preferably greater than the number of alkoxy groups, the electron withdrawing nature of the oxidized sulfur-containing groups is expected to affect the properties of the handle to a greater degree. The alkoxy groups are advantageous for cleaving the handle after reduction of the sulfur-

containing groups because of their electron donating properties.

It is believed that the stability of the oxidized handle and the acid lability of the reduced handle are enhanced by increasing the number of sulfur-containing substituents on one or both benzene rings. Thus the increased electron withdrawing nature of a handle with more than one oxidized sulfur-containing group may result in greater chemical stability of the handle. Similarly, the increased electron donating potential of more than one reduced sulfur-containing groups may facilitate cleavage under mild conditions.

Additionally, oxidation of the sulfur-containing group to yield a sulfone is expected to result in a more stable handle than oxidation to the sulfoxide because the sulfone has greater electron withdrawing properties. Since either the sulfone or the sulfoxide can be reduced to yield a sulfide, either oxidation state is suitable for handles of the invention. Presently, the sulfoxide oxidation state is preferred.

In a further embodiment, the handle can be prepared with a protecting group at the amine nitrogen. Suitable protecting groups include tert-butoxycarbonyl (Boc) and N-9-fluorenylmethyloxy carbonyl (Fmoc), to name a few. Any amine protecting group suitable for peptide synthesis is suitable. Many are described in Greene and Wuts, Protective Groups in Organic Synthesis, Wiley Interscience: New York, 1991, pp. 309-362; and Fields and Noble 1990, Int. J. Pept. Protein Res. 35: 161-214. The N-protected benzhydrylamine can be coupled to the solid support without concern for reactivity of the amine group. After the handle is coupled to the solid support, the protecting group can be removed by routine chemistry, e.g., treatment with piperidine in

dimethylformamide in the case of Fmoc, or treatment with trifluoroacetic acid in the case of Boc.

In another embodiment, the handle can be prepared as a benzhydrylamide, i.e., with an acyl group on the amine. Acyl groups for use in the invention include but are not limited to N<sup>α</sup>-protected amino acids, which can be deprotected for subsequent elongation of a peptide chain. The term amino acids is used here as defined above, and includes D and L amino acids, glycine, and non-naturally occurring amino acids or amino acid analogs.

When X (or Z) is found as a sulfoxide or sulfone group on the handle, the handle is extremely resistant to acidic media. Reduction of a sulfoxide or sulfone to a sulfide yields a handle that is labile to acidolysis. For example, such a handle can be cleaved in trifluoroacetic acid. Thus, the present invention provides handles that are acid stable or acid labile, and provides for conversion of the acid stable form to the labile form, and vice versa.

#### 4.2. SYNTHESIS OF SUBSTITUTED BENZYHDRYLAMINES

The substituted benzhydrylamines of the present invention can be prepared according to the following methods.

##### 4.2.1. PREPARATION OF BENZOPHENONES

Substituted benzophenones for use as starting materials for synthesis of handles of the invention can be prepared synthetically, for example by a condensation reaction of a substituted benzoic acid with a substituted benzene, i.e., acylation of benzene.

Suitable substituted benzoic acids include but are not limited to a 2-alkylthiobenzoic acid, 4-alkylthiobenzoic acid, 2,4-di(alkylthio)benzoic acid, 2,4,6-tri(alkylthio)benzoic acid, 2-(or 4-) alkylthio-4(or 2-)alkoxybenzoic acid, 2,4-(or 2,6-alkylthio)-6(or 4)-alkoxybenzoic acid, and the like. Moreover, the benzoic acid may be substituted at the 2,3 or 4 position with an alkyl group comprising a functional group for coupling to the solid phase support (i.e., Z). Special care must be taken to protect the functional group present on the alkyl group to prevent its reaction during formation of the benzophenone. For example, if the functional group is a carboxylic acid, it must be protected from reaction with thionyl chloride. Alternatively the benzoic acid can be substituted at the 2, 3 or 4 position with a hydroxy or mercapto (i.e., sulfhydryl) group. The hydroxy or mercapto group is preferably at the 2 or 4 position. If the substituted benzoic acid includes a 2 or 4 mercapto substituent, it need not include an alkylthio substituent since upon alkylation, the mercapto group will become an alkylthio group with the required properties. In a specific embodiment, the substituted benzoic acid is 2-hydroxy-4-(methylthio)benzoic acid.

Suitable substituted benzene groups include but are not limited to alkylthiobenzene (e.g., thioanisole), 1,3-dialkylthiobenzene, 1,3,5-trialkylthiobenzene, 3-alkoxy-alkylthiobenzene (e.g., 3-alkoxythioanisole), 5-alkoxy-1,3-dialkylthiobenzene and 3-alkoxy-1,5-dialkylthiobenzene. In a specific embodiment, the substituted benzene is thioanisole.

In a further embodiment, if the benzoic acid lacks an alkyl group for use as a linker, and lacks a hydroxy or mercapto group suitable for alkylation to

attach the linker, the substituted benzene can include either of these groups.

Many variations are possible for the substituted benzoic acid and substituted benzene that can be used to prepare the benzophenone. Although many combinations and permutations are possible, and are contemplated by the instant invention, often the simplest starting materials are preferred, since these are generally less expensive, easier to obtain, and less susceptible to undesirable side reactions. Thus, the substituted benzoic acid and substituted benzene chosen must form a benzophenone with at least one sulfur-containing substituent in a para or ortho position on a benzene ring, preferably a sulfur-containing substituent on both benzene rings, and a linker or a functional group suitable for attachment of a linker.

In a specific embodiment, infra, a substituted benzoic acid is heated in thionyl chloride to yield the corresponding benzoic acid chloride. Generally this reaction can be run by heating the benzoic acid in about two molar equivalents of thionyl chloride to about 40°C for about 10 min to about 30 min with stirring. The benzoyl chloride is then dissolved (at about 0.1 to about 1M) in an aprotic solvent, e.g., ethylene dichloride, at about 40°C. This solution is mixed with an equimolar amount of a substituted benzene, e.g., thianisole, dissolved at about 0.2 to about 3M in an aprotic solvent, (e.g., dichloroethane). The mixture is cooled to 0°C, and treated with an equivalent of aluminum chloride. The reaction mixture is allowed to warm to about 20°C, at which point a second equivalent of AlCl<sub>3</sub> can be added while temperature is controlled. The reaction mixture is stirred for about 1 to 2 hours at about 40°-50°C,

and worked up by standard methods, e.g., extraction, recrystallization, etc.

It is also contemplated that a benzophenone can be modified by addition of sulfur-containing groups to the ortho or para positions, or by substitution of sulfur for oxygen in alkoxy-substituted benzophenones. The exchange of a sulfide for an alkoxy group occurs by treatment of the hydroxy group with chlorodimethylthiocarbamate followed by thermal rearrangement and S-alkylation. This alternative, however, requires use of an asymmetrically substituted benzophenone to provide for attachment of, or alternatively to act as, a single linker for attachment to the solid phase support.

#### 4.2.2. ALKYLATION OF HYDROXY OR MERCAPTO-SUBSTITUTED BENZOPHENONES

In the case in which the benzophenone is prepared without an alkyl linking group, alkylation to add the linking group is required. Any alkylation procedure known in the art can be used in the practice of the instant invention. These alkylation procedures are accomplished by activation of an alkyl with a halogen, especially bromine or chlorine, or with another good leaving group. However, where alkylation at the ortho position is contemplated, an agent must be added to disrupt hydrogen bonding. In a preferred embodiment, the agent is fluoride ion (F<sup>-</sup>).

In a specific embodiment, a substituted benzophenone is dissolved in a polar aprotic solvent. The benzophenone is substituted with a hydroxy or mercapto (SH) group at the 2, 3 or 4 position on one benzene ring. Preferably the concentration of the benzophenone is 5 mM to 1 M, more preferably 50 mM to 0.5 M. The optimal concentration depends on the



choice of polar aprotic solvent, and the solubility of the benzophenone therein. Suitable solvents include but are not limited to acetonitrile, dimethyl  
5 formamide, dioxane, and the like, as well as mixtures thereof.

When the hydroxy or mercapto group is in the 2-position, fluoride ions are present in the solution to facilitate the alkylation reaction. The ions can be  
10 provided on an inert support. In a specific embodiment, infra, the fluoride is provided as a suspension of potassium fluoride on alumina. In another embodiment, fluoride is provided as tetraethylammonium fluoride.

15 To this solution is added an alkylating agent, e.g., an  $\omega$ -haloester of alkylcarboxylic acid, which can be prepared by halogenation of the corresponding commercially available alkylcarboxylic acid. In a specific embodiment, methyl bromovalerate is added.  
20 The alkylating agent is preferably added in molar excess relative to the concentration of the benzophenone, preferably greater than about a 20% excess, more preferably greater than about a 40% excess. The alkylation reaction can require at least  
25 about 96 hours for completion at room temperature. The alkylated benzophenone can be purified by routine methods, e.g., chromatography or crystallization, or it can be separated from solid materials (e.g., if alumina is present) concentrated, e.g., in vacuo, and  
30 used without further purification.

#### 4.2.3. CONVERSION OF THE BENZOPHENONE TO A BENZHYDRYLAMINE

The carbonyl group of the substituted  
35 benzophenones is converted to an amine group. Conversion of a carbonyl to an amine can be

accomplished by any method known in the art, including but not limited to oxime formation followed by reduction, or reductive amination, e.g., with ammonium formate. In a specific embodiment, infra, the benzophenone is reacted with hydroxylamine to yield an oxime. This reaction is generally performed in a polar solvent, preferably an alcohol, e.g., ethanol, although methanol, propanol, 2-propanol, etc., can also be used with the substituted benzophenone at roughly the same concentration as is used for the alkylation reaction. Anhydrous weak base, e.g., sodium acetate, can also be present. The reaction mixture is heated to reflux for about 1 to about 15 hours, preferably about 7 hours. The mixture can be left to stand, e.g., overnight, or worked up immediately. In work-up the reaction mixture can be filtered and concentrated in vacuo to yield a product that can be used without further purification.

The oxime product is dissolved in alcohol (preferably ethanol) and 25% ammonia at about a 1:3 ratio in about the same concentration as the alkylated benzophenone was used in the oxime step. To this solution is added about a 10-fold molar excess of powdered zinc. The suspension is heated to 50°C and stirred for about 48 hours. The suspension is filtered and concentrated in vacuo to yield the substituted benzhydryl amine.

The benzyhydrylamine obtained by reduction of the oxime is sparingly soluble or insoluble in polar solvents. Moreover, when the reduction reaction is performed over zinc, zinc remains in the reaction mixture. Thus prior to oxidation of the sulfur-containing substituents (alkylsulfides) and protection of the amino group, it is necessary to purify the benzyhydrylamine. Purification procedures include but

are not limited to chromatography, e.g., on silica gel, recrystallization, preparative thin layer chromatography and the like.

5        In a specific embodiments, a recrystallization procedure is used. The insoluble benzhydrylamine is solubilized by adding toluene sulfonic acid, thus forming the acid salt of the amine. This reaction can be performed by dissolving the benzhydrylamine in an  
10 appropriate solvent, e.g., methanol, ethanol, and the like, preferably ethanol, at about 100-500 mM, preferably at about 150 mM. A saturated solution of toluenesulfonic acid (TsOH) in the solvent can be added, preferably by a controlled addition, e.g.,  
15 dropwise addition. The product is dissolved in a polar, protic solvent system that includes water (e.g., an alcohol, such as methanol, and water, 3:1 v/v), and if necessary heated, to increase solubility. After the solution has cooled to about 20°C, an  
20 oxidizing agent, e.g. sodium periodate (to form the sulfoxide) or hydrogen peroxide (to form the sulfone) is added in molar excess based on equivalents of S atoms. Typically the solvent for oxidation with hydrogen peroxide is glacial acetic acid. Precipitate  
25 formed during the sodium periodate oxidation is removed by filtration and the product concentrated.

#### 4.2.4. PROTECTION OF THE AMINE GROUP

30        The present invention further provides for protection of the amine group after synthesis of the benzhydrylamine. Protection of the amine group can be important to prevent reactivity of the amine when coupling the handle to the solid support. Thus the  
35 invention provides amine-protected handles.

Preferred protecting groups for the amine are Boc and Fmoc. However, the other protecting groups, such as are described in Greene and Wuts, Protective Groups in Organic Synthesis, Wiley Interscience: New York, 1991, pp. 309-362; and Fields and Noble 1990, Int. J. Pept. Protein Res. 35: 161-214 are also contemplated. The synthesis of Boc and Fmoc protected amines is straightforward and well known in the art. N<sup>α</sup>-protected amino acids for peptide synthesis are usually protected with Boc or with Fmoc, depending on the synthetic strategy.

In a specific embodiment, infra, the amine is protected with an Fmoc protecting group. The pH of the aqueous solution containing benzhydrylamine is brought to about 9, any inorganic salts that have precipitated are removed by filtration, and protected Fmoc (e.g., Fmoc-H-N-hydroxy-succinimidyl ester) added. The Fmoc-benzhydrylamine is worked up using standard procedures to yield a product of desired purity.

In an alternative embodiment, the benzhydrylamine is protected with the Boc protecting group. Generally, this can be accomplished according to routine methods.

#### 4.2.5. ALTERNATE ROUTE FOR PREPARING THE HANDLE

A benzophenone is prepared as described in Section 4.2.1., supra. The benzophenone can be reduced, e.g., by treatment with sodium borohydride in ethanol or other similar solvent, to yield benzhydryl alcohol. Treatment of the benzhydryl alcohol with a primary amide under acidic conditions results in substitution of the amide for the hydroxy group. This result is analogous to an acylation of a benzhydrylamine.

Preferably the amide group that is chosen is an N<sup>α</sup>-protected amino acid, as described in Section 4.1., supra.

5

#### 4.3. USE OF THE HANDLE FOR PEPTIDE SYNTHESIS

The handles of this invention are well suited for attaching a peptide chain to a solid phase support for peptide synthesis. The handles can be attached to any amino resins via a suitable functional group, e.g., carboxylic acid, on the alkyl group. Attachment of a carboxylic acid functional group to an amine on the resin can proceed according to any of the techniques commonly used for peptide synthesis, e.g., preparation of an OPfp, HOBt or other activated ester, condensation in the presence of a carbodiimide, etc. These methods are also discussed for synthesis of peptides in Section 4.3.2. Suitable solid supports for use in the invention are discussed, supra.

20 Solid phase peptide synthesis techniques are well known in the art. Simply put, an N<sup>α</sup>-protected amino acid is activated at the α-carbonyl and coupled with the deprotected N<sup>α</sup> of the nascent peptide-handle-solid phase support. The coupling reactions may be accomplished by techniques familiar to those in the art (see, e.g., Stewart and Young, 1984, Solid Phase Synthesis, Second Edition, Pierce Chemical Co., Rockford, IL; Fields and Noble, 1990, "Solid phase peptide synthesis utilizing 9-fluorenylmethyloxy-carbonyl amino acids," Int. J. Pept. Protein Res. 35:161-214; Geysen et al., 1987, J. Immunol. Methods 102:259-274). The chemistry of coupling, deprotection, and finally cleavage of the peptide from the solid phase support depends on choice of N-protecting group, which is generally tert-

35

butoxycarbonyl (Boc) or 9-fluorenylmethyloxycarbonyl (Fmoc).

In a preferred embodiment of peptide synthesis  
5 using handles of the invention the completeness of  
coupling should be assessed. Methods of assessing the  
completeness of coupling are well known in the art.  
If the coupling was not complete, the reaction should  
be forced to completion by a second coupling, e.g.,  
10 (a) by using a higher concentration of the activated  
amino acid or a different activation mechanism; (b)  
with the addition of different or additional solvents;  
or (c) with the addition of chaotropic salts (see Klis  
and Stewart, 1990, in Peptides: Chemistry, Structure  
15 and Biology, Rivier and Marshall (eds.), EDSCOM  
Publishers, pp. 904-906).

#### 4.3.1. REDUCTION AND CLEAVAGE

After peptide synthesis, the peptide must be  
20 cleaved from the solid phase support. If the handle  
of the invention is in its reduced form, cleavage is  
accomplished by treatment with acid, for example TFA.  
Where Fmoc amino acid strategy has been employed, the  
cleavage will also result in deprotection of the side  
25 chains.

Alternatively, when the handle has been used in  
the oxidized form, either sulfoxide or sulfone, the  
handle can be reduced to convert it to acid labile  
handle. Reduction agents known in the art can be  
30 used. For example, about 1M trimethylsilylchloride/  
triphenylphosphine/tetrahydrofuran or more preferably  
1M trimethylsilylchloride/triphenylphosphine/  
dichloromethane can be used to reduce the sulfoxide to  
the sulfide. Treatment with samarium-iodide ( $\text{SmI}_2$ )  
35 will reduce the sulfone and sulfoxide to the sulfide.  
In yet a further embodiment, the reduction,

deprotection and cleavage can be accomplished simultaneously by treatment with silyl bromide. In another embodiment, reduction, deprotection (of Fmoc amino acids) and cleavage can be accomplished simultaneously by treatment with 1M trimethylsilylbromide/thioanisole/trifluoroacetic acid.

A particular advantage of the instant invention is that the amino acid side chains can be deprotected prior to cleavage. In a specific embodiment, *infra*, the deprotected side chains react to form a cyclic peptide prior to cleavage (see Sections 7.3 and 8., *infra*). By cyclizing prior to cleavage, side reactions and intermolecular bonding is avoided.

5. EXAMPLE: PREPARATION OF A SOLID SUPPORT WITH A N-FMOC [2-(CARBOXYLATOBUTYL-4-OXY)-4-(METHYLSULFINYL) PHENYL]-[4'-(METHYLSULFINYL) PHENYL] METHYLAMINE

5.1. 4.4'-BIS(METHYLTHIO)-2-HYDROXYBENZOPHENONE

A solution of 2-hydroxy-4-methylthiobenzoic acid (45.00 g, 227 mmol) in thionyl chloride (50 ml, 685 mmol) was heated at 40°C for 15 min with stirring. After addition of 1,2-dichloroethane (EDC) (90 ml) the solution was stirred at 50°C for 30 min under vacuum (15 mm).

The solid residue was dissolved in EDC (250 ml) at 40°C and added to the solution of thioanisole (26.7 ml, 227 mmol) in EDC (100 ml). The resulting mixture was cooled to 0°C and finely ground AlCl<sub>3</sub> (33.0 g, 247 mmol) was added portionwise within 75 min at 0-5°C. The mixture was allowed to warm to 20°C and the second equivalent of AlCl<sub>3</sub> (33.7 g, 253 mmol) was added within 30 min (caution: the temperature rises to 30°C and the mixture must be cooled). When addition of AlCl<sub>3</sub> was finished, the reaction mixture was stirred at 45-

50°C for 1.5 hr, then cooled down to 20°C and poured into mixture of ice (300 g) and conc. HCl (180 ml). The organic phase was evaporated and the aqueous layer  
5 was heated at reflux for 15 min, cooled down to 20°C and extracted with EDC (4 X 150 ml). The collected organic phases were washed with water (3 x 50 ml), 10% NaHCO<sub>3</sub> (2 x 150 ml), water (1 x 150 ml), 1M HCl (1 x 150 ml) and water (2 x 150 ml) and then were  
10 dried over MgSO<sub>4</sub>. Removal of the solvent in vacuo gave yellow crystals which were recrystallized from EtOH (2100 ml) to give a yellow product (41.00 g, 62%), m.p. 104-106°C. Anal. Calcd for C<sub>15</sub>H<sub>14</sub>O<sub>2</sub>S<sub>2</sub> (290.41); C 62.04%, H 4.86%, S 22.08%. Found: C 61.86%, H 4.78%,  
15 S 22.06%. <sup>1</sup>H NMR (CDCl<sub>3</sub>): 2.51s (3H, SCH<sub>3</sub>); 2.54s (3H, SCH<sub>3</sub>); 6.60-6.85m (2H, aromatic); 7.26-7.66m (5H, aromatic); 12.35s (1H, OH). MS-EI: 290(M<sup>+</sup>, 100); 275(20); 243(30); 228(10); 167(40); 151(30); 124(50); 105(10); 77(10); 57(10); 45(10). IR (CCl<sub>4</sub>): ν<sub>co</sub>  
20 1620cm<sup>-1</sup>; ν<sub>OH</sub> 3000 cm<sup>-1</sup>.

5.2. 5-[3-METHYLTHIO-1-OXY-5-(4-METHYLTHIOBENZOYL)PHENYL]-PENTANOIC ACID METHYLESTER

25 A suspension of alumina (90 g, Fluka-Type 504 C, acid type) and potassium fluoride (dihydrate) (60 g) in water (600 ml) was evaporated to dryness in vacuo. The residue was dried at 135-140°C for 24h to afford 120 g of KF/Al<sub>2</sub>O<sub>3</sub>.

30 4,4'-Bis(methylthio)-2-hydroxybenzophenone (50.54 g, 174 mmol) was dissolved in acetonitrile (800 ml) at 60°C. The solution was rapidly cooled down to 25°C and KF/Al<sub>2</sub>O<sub>3</sub> (127 g) was added all at once. To the stirred suspension methyl bromovalerate (32.4 ml, 226  
35 mmol) was added portionwise within 90 hr and stirring was continued for 120 hr at which time RP HPLC



analysis indicated the reaction was complete (Vydac C-18 column, isocratic eluent 75% MeOH, 0.1% TFA, starting compound  $R_t$  = 16 min, product  $R_t$  = 7 min). The suspension was filtered, filtrate cake washed with acetone (2 x 50 ml) and the filtrate concentrated in vacuo to give yellow product which was used in the next step without further purification. Analytical sample: m.p. 61-63°C (EtOH);  $R_t$  0.45 (petroleum ether:EtOAc - 60:40). Anal. 61-63°C (EtOH);  $R_t$  0.45 (petroleum ether:EtOAc - 60:40). Anal. Calcd for  $C_{21}H_{24}O_4S_2$  (404.55): C 62.35%, H 5.98%, S 15.85%. Found: C 62.20%, H 6.02%, S 15.62%.  $^1H$  NMR ( $CDCl_3$ ): 1.25-1.55m (4H, 2 x  $CH_2$ ); 2.15t (2H,  $CH_2COO$ ,  $J^3$  = 7.5 Hz); 2.52s (3H,  $SCH_3$ ); 2.53s (3H,  $SCH_3$ ); 3.64s (3H,  $COOCH_3$ ); 3.90t (2H,  $O_4CH_2$ ,  $J^3$  = 5Hz); 6.79d (1H,  $J^4$  = 1.5 Hz, aromatic); 6.88dd (1H,  $J^3$  = 8 Hz,  $J^4$  = 1.5 Hz, aromatic); 7.18 - 7.25 m (2H, aromatic); 7.36d (1H,  $J^3$  = 8 Hz,  $J^4$  = 1.5 Hz, aromatic); 7.18 - 7.25m (2H, aromatic); 7.36d (1H,  $J^3$  = 8 Hz, aromatic); 7.64 - 7.71m (2H aromatic) EI-MS: 404 ( $M^+$ , 30); 289(20); 227(40); 167(35); 151(35); 124(20); 115(100); 83(20); 55(40).

5.3. 5-[3-METHYLTHIO-1-OXY-5-(4-METHYLTHIOBENZOYL)PHENYL]-PENTANOIC ACID

The above prepared crude 5-[3-methylthio-1-oxy-5-(4-methylthiobenzoyl)phenyl]pentanoic acid methylester was dissolved in dioxane (650 ml) and to this solution, 4M NaOH (131 ml, 522 mmol) and methanol (200 ml) were added. The resulting clear solution was stirred overnight at room temperature and then the organic solvents were evaporated in vacuo. The aqueous solution was extracted with ethyl acetate (EtOAc) (2x100 ml) and the aqueous layer was kept in vacuo for 30 min to remove traces of EtOAc. The

product was precipitated by slow addition of 20% H<sub>2</sub>SO<sub>4</sub> (to pH 2), washed with water and dried over P<sub>2</sub>O<sub>5</sub> in vacuo to give an off-white powder (50.18 g, 87%, based on starting 4,4'-bis(methylthio)-2-hydroxybenzo-phenone). Analytical sample: m.p. 113-114°C (EtOH); R<sub>f</sub> 0.22 (petroleum ether:EtOAc-60:40). Anal. Calcd for C<sub>20</sub>H<sub>22</sub>O<sub>4</sub>S<sub>2</sub> (390.52): C 61.55%, H 5.68%, S 16.42%. Found: C 61.15%, H 5.79%, S 16.39%. EI-MS: 390 (M<sup>+</sup>, 50); 290(90); 243(50); 227(40); 167(100); 151(70); 124(95), 55(60).

5.4. 2-(CARBOXYLATOBUTYL-4-OXY)-4-METHYLTHIO-4'-METHYLTHIOBENZOPHENONE OXIME

A suspension of 5-[3-methylthio-1-oxy-5-(4-methylthio-benzoyl)phenyl]pentanoic acid (18.00 g, 46.1 mmol), anhydrous sodium acetate (9.45 g, 115 mmol) and hydroxylamine hydrochloride (9.60 g, 138 mmol) in 96% ethanol (240 ml) was heated at reflux for 7 hr. After standing overnight at room temperature, the mixture was filtered and concentrated in vacuo to give a white solid, which was used without further purification in the next step. Analytical sample: m.p. 166-170°C (EtOH, isomeric mixture); R<sub>f</sub> 0.41 (CHCl<sub>3</sub>:eOH-10:1). EI-MS: 405(M<sup>+</sup>, 2), 387 (25); 300(45); 288(100); 242(30); 227(20); 168(30); 151(40); 124(10); 55(35).

5.5. [2-(CARBOXYLATOBUTYL-4-OXY)-4-(METHYLTHIO)PHENYL-4'-METHYLTHIO-PHENYL]METHYLAMINE 4-TOLUENESULFONATE

Crude 2-(carboxylatobutyl-4-oxy)-4-methylthio-4'-methylthio-benzophenone oxime (minimum 46.1 mmol) prepared above was placed in a 500 ml Champagne bottle together with powdered zinc (18.37 g, 461 mmol), ethanol (80 ml) and 25% ammonia (240 ml). The

suspension was magnetically stirred at 50°C for 48 hr. After filtration, the filtrate was concentrated in vacuo to give a white solid. After addition of water (300 ml) pH was adjusted to 7 with a saturated solution of toluene sulfonic acid (TsOH) in EtOH and the suspension was filtered. The off-white product was washed with water and then suspended in ethanol (300 ml) at about 60°C. To this suspension, the saturated solution of TsOH in EtOH was added dropwise until the mixture turned to a clear orange solution. Standing in a refrigerator for 48 hr afforded pink crystals, which were suspended by filtration to give the product 1 (18.2 g, 70%), m.p. 178-180°C (dec);  $R_f$  0.58 (n-BuOH:AcOH:H<sub>2</sub>O-80:20:20). FAB-MS: 392(m+1); 375; 275; 227; 137. Anal calcd for C<sub>27</sub>H<sub>33</sub>NO<sub>6</sub>S<sub>3</sub> (563.76); C 57.52%, H 5.9%, N 2.48%, S 17.06%. Found: C 57.87%, H 5.67%, N 2.40%, S 17.11%.

5.6. N-9-Fmoc-([2-CARBOXYLATO-BUTYL-4-OXY)-4-(METHYLSULFINYL)PHENYL-4'-(METHYLSULFINYL)-PHENYL]METHYLAMINE

The [2-(carboxylatobutyl-4-oxy)-4-(methylthio)phenyl-4'-methylthiophenyl]methylamine 4-toluene-sulfonate (20.00 g, 35.5 mmol) was suspended in the mixture of methanol (320 ml) and water (160 ml). The suspension was heated until the solution became clear, then chilled to 20°C and a solution of sodium periodate (15.18 g, 71 mmol) in water (140 ml) was added dropwise over a period of 1 hr. The stirring was continued further for 2 hr. The sodium iodate which separated was removed by filtration and the methanol was evaporated in vacuo. The pH of remaining aqueous solution was adjusted to 9 by addition of 4 M NaOH and the solution was mixed with acetonitrile (200 ml). The mixture was filtered to remove precipitated

inorganic salts and a solution of fluorenylmethyloxy-carbonyl-N-hydroxysuccinimide (Fmoc-succinimide) (12.99 g, 38.5 mmol) in acetonitrile (80 ml) was added rapidly. The resultant homogenous solution was stirred at 25°C while adjusting the pH to 8.5-9 with 1M NaOH. After 2 hr no further pH change occurred and the acetonitrile was removed in vacuo. The remaining aqueous solution was diluted with water (100 ml) and extracted with ether (4 x 50 ml, discarded). The aqueous part was mixed with chloroform (150 ml) and 1M H<sub>2</sub>SO<sub>4</sub> was added with occasional shaking until the aqueous phase reached pH 2. The aqueous phase was then extracted with chloroform (4 x 50 ml). The combined organic phases were washed with water (100 ml), dried (MgSO<sub>4</sub>), and concentrated to give an oil which after dissolving in methanol (150 ml) and standing overnight in refrigerator afforded the product as a white solid. Concentration of mother liquor and addition of ethyl acetate (50 ml) gave the second portion of the product. Yield 14.75g (65%), m.p. - 135-137°C. R<sub>f</sub> (CHCl<sub>3</sub>:MeOH-9:1) = 0.11. <sup>1</sup>H NMR (CDCl<sub>3</sub>): 1.3-1.6m (4H, CH<sub>2</sub>), 2.17t (2H, CH<sub>2</sub>COO, J=8 Hz), 2.75s (3H, SOCH<sub>3</sub>), 2.76s (3H, SOCH<sub>3</sub>), 3.8-4.05m (2H, OCH<sub>2</sub>), 4.22t (1H, Fmoc-CH, J=8 Hz), 4.4-4.6m (2H, CH<sub>2</sub>-Fmoc), 5.95d (1H, Ar<sub>2</sub>CH, J=9 Hz), 6.25d (1H, NH, J=9 Hz), 7.10-7.80m (15H, aromatic). Anal Calcd for C<sub>35</sub>H<sub>35</sub>NO<sub>7</sub>S<sub>2</sub> (645.8): C 68.10%, H 5.46%, N 2.17%, S 9.93%. Found: C 68.55%, H 5.50%, N 2.11%, S 9.89%.

#### 6. EXAMPLE: ATTACHMENT OF HANDLE ONTO SUPPORT

The solution on N-Fmoc-{[2-(carboxylatobutyl-4-oxy)-4-(methylsulfinyl)phenyl]-4'-(methylsulfinyl)-phenyl}methylamine (245 mg, 0.38 mmol), N-hydroxybenzotriazole (52 mg, 0.38 mmol), 4-dimethylaminopyridine (5 mg, 0.038 mmol), and N,N'-diisopropyl-

carbodiimide (50  $\mu$ l, 0.38 mmol) in dimethylformamide (10 ml) was added to p-methylbenzhydrylamine (pMBHA) resin (200 mg, 0.076 mmol) (Peptides International, Louisville, KY) and the suspension was shaken overnight. The resin was washed with dimethylformamide (5 x 5 ml) and allowed to react with the solution of acetic acid (46  $\mu$ l, 0.76 mmol) and N,N'-diisopropylcarbodiimide (119  $\mu$ l, 0.76 mmol) in dimethylformamide (10 ml) for 5 hr to acetylate the remaining free amino groups. After washing with dimethylformamide (5 x 5 ml), dichloromethane (5 x 5 ml), and methanol (5 x 5 ml) the resin was dried in vacuo. The substitution estimated spectrophotometrically, was 0.34 mmol/g.

7. EXAMPLE: PREPARATION OF PEPTIDES USING THE HANDLE

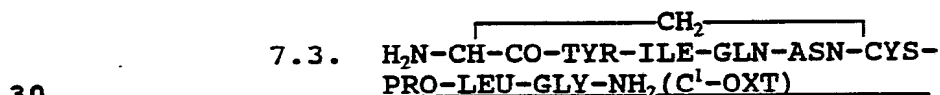
7.1. H-PHE-PRO-GLN-THR-ALA-ILE-GLY-VAL-GLY-ALA-PRO-NH<sub>2</sub>

The above prepared resin (132 mg) was used. The general synthetic protocol was as follows: The Fmoc protecting group on the handle was removed with piperidine/dimethylformamide (1:1, v/v, 1 x 3 min, 1 x 10 min) followed by washing with dimethylformamide. Boc protecting groups were removed with mixture of trifluoroacetic acid/dichloromethane/anisole (50:50:1, 1 x 3 min, 1 x 25 min) followed by washing with dichloromethane. N-Hydroxybenzotriazole active esters were used throughout all synthesis (10 min pre-activation). The Boc protecting group was used in the cases of Gly, Val, and Ile amino acids. In the other cases, the Fmoc group was used. The coupling was monitored with bromophenol blue (Krchnak et al., 1988, Collect. Czech. Chem. Commun. 53:2542). Final cleavage from support was accomplished with the

mixture of 1M trimethylsilylbromide/thioanisole/  
trifluoroacetic acid (2 hr, 0°C) followed by dilution  
with water and extraction with diethyl ether. The  
5 crude peptide was obtained in 95% yield (43 mg) and  
was purified on Sephadex G 25 with 1 M acetic acid as  
eluent. After preparative reverse phase HPLC the pure  
peptide was obtained in 62% yield (26.6 mg). FAB-MS:  
1056 (M+1), 942, 871, 814, 715, 658, 545, 474, 391,  
10 245, 217, 120. Amino acid analysis: Thr (1.00), Gln  
(1.02), Pro (1.97), Gly (2.00), Ala (1.95), Val  
(0.97), Ile (0.94), Phe (1.01).

7.2. BOC-TYR-ILE-GLN-ASN-CYS(ClZ-ABU  
-O<sup>t</sup>BU)-PRO-LEU-GLY-HANDLE-pMBHA RESIN

15 pMBHA resin prepared analogously as in Example 6  
(400 mg, 0.052 mmol) was used for the synthesis of C<sup>1</sup>-  
OXT. After each coupling, the remaining free amino  
groups were acetylated with the mixture of acetic  
20 acid/N-hydroxybenzotriazole/N,N'-diisopropyl-  
carbodiimide/N-methylimidazole. The general synthetic  
protocol used was the same as described in 8.1, supra.  
All amino acids used were Fmoc-derivatives. Amino  
acid analysis: Asp(0.98), Glu(1.00), Pro(0.94),  
25 Gly(1.13), Cyth(0.54), Ile(0.90), Leu(1.04),  
Tyr(0.75).



Above prepared peptidyl-resin (Section 7.2,  
supra) (100 mg, 0.012 mmol) was washed with  
dichloromethane (3 x 5 ml) and tert-butyl protecting  
groups were removed with mixture of trifluoroacetic  
35 acid/dichloromethane/anisole (50:50:1, 1 x 3 min, 1 x  
25 min), followed by washing with dichloromethane (5 x

5 ml), neutralized with 7% N,N'-diisopropylcarbodiimide/dichloromethane, washed with dichloromethane (3 x 5 ml) and finally washed with  
5 N,N-dimethylformamide (3 x 5 ml). The cyclization was carried out on the support with N-hydroxybenzotriazole/N,N'-diisopropylcarbodiimide/N,N-dimethylformamide mixture for 24 hr to give a cyclized, fully protected peptide. The final cleavage  
10 was accomplished with 1 M trimethylsilylbromide/thioanisole/trifluoroacetic acid mixture containing 5% of m-cresole (2 hr, 0°C). The peptide was precipitated with diethyl ether, filtered, washed with diethyl ether and purified on Sephadex G 25 (1 M  
15 acetic acid) to give 10.6 mg (89%) of crude product, which was purified on preparative RP-HPLC (Vydac C18) to give 4.5 mg (38%) of a pure C<sup>1</sup>-OXT. Amino acid analysis: Asp(0.99), Glu(1.00), Pro(0.95), Gly(0.99), Cyth(1.02), Ile(0.96), Leu(1.10), Tyr(0.93),  
20 MS(FAB)=990 (M+H).

8. EXAMPLE: TWO-STEP DEPROTECTION OF C<sup>1</sup>-OXT PRECURSOR

Above prepared peptidyl-resin (Section 7.2,  
25 supra) (100 mg, 0.012 mmol) was treated with the mixture of 1M trimethylsilylchloride/triphenylphosphine/tetrahydrofuran (2 ml) at 25°C for 2 hr. Then the resin was washed with dichloromethane (5 x 5 ml) and the peptide was cleaved from the resin with  
30 the mixture of trifluoroacetic acid/m-cresole (95:5) (2.5 ml) at 25°C for 30 min. After evaporation to dryness, the peptide was precipitated with diethyl ether to give a powder which was purified on Sephadex G 25 (1 M acetic acid) followed by preparative RP-HPLC  
35 (Vydac C18) to give 6.5 mg (43%) of a pure H<sub>2</sub>N-Tyr-Ile-Gln-Asn-Cys(ClZ-Abu-OH)-Pro-Leu-Gly-NH<sub>2</sub>. Amino acid

analysis: Asp(1.04), Glu(1.04), Pro(0.95), Gly(1.03),  
Cyth(0.94), Ile(0.88), Leu(1.11), Tyr(0.85),  
MS(FAB)=1176 (M+H).

5

The present invention is not to be limited in  
scope by the specific embodiments described herein.  
Indeed, various modifications of the invention in  
addition to those described herein will become  
10 apparent to those skilled in the art from the  
foregoing description and accompanying figures. Such  
modifications are intended to fall within the scope of  
the appended claims.

Various publications are cited herein, the  
15 disclosures of which are incorporated by reference in  
their entireties.

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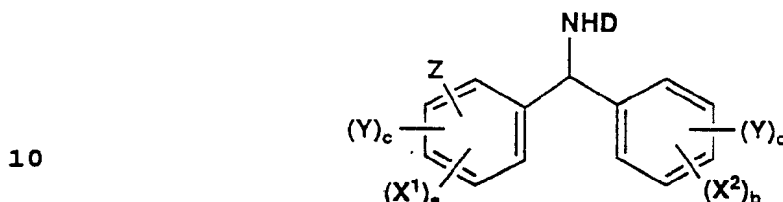
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What is claimed is:

1. A handle for use in peptide synthesis of  
5 the general formula (I):



15 in which a = 1 to 3, X<sup>1</sup> is [S]R<sup>1</sup>, or X<sup>1</sup> is Z, X<sup>1</sup> groups are in the ortho or para positions of the first benzene ring, and R<sup>1</sup> is an alkyl group; and

in which b = 1 to 3, X<sup>2</sup> is [S]R<sup>2</sup>, X<sup>2</sup> groups are in the ortho or para positions of the second benzene ring, and R<sup>2</sup> is an alkyl group; and

20 in which Z is R<sup>3</sup>, OR<sup>3</sup> or [S]R<sup>3</sup> in any position not occupied by X<sup>1</sup> unless X<sup>1</sup> is Z, and R<sup>3</sup> is an alkyl group comprising a reactive functional group for coupling to a solid phase support, in which if X<sup>1</sup> is Z, Z is [S]R<sup>3</sup> in an ortho or para position; and

25 in which c = 0 or 1 and d = 0 or 1, a Y group is in an ortho or para position not occupied by X<sup>1</sup> or X<sup>2</sup>, Y is OR<sup>4</sup>, and R<sup>4</sup> is an alkyl group; and

in which [S] is -S-, -SO- or -SO<sub>2</sub>-; and

in which D is H, a protecting group or an N<sup>α</sup>-protected acyl.

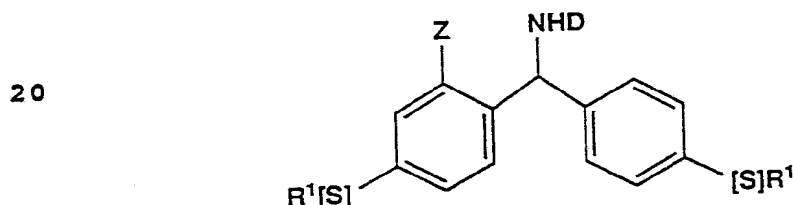
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2. The handle of claim 1 in which a = 1, X<sup>1</sup> is in the para position, b = 1, and X<sup>2</sup> is in the para position.

35

3. The handle of claim 1 in which Z is OR<sup>3</sup> in the ortho position.

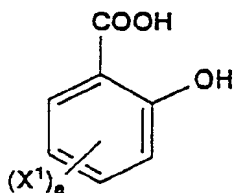
4. The handle of claim 1 in which [S] is -S-.
5. The handle of claim 1 in which S is -SO- or  
5 -SO<sub>2</sub>-.
6. The handle of claim 1 in which P is a protecting group.
- 10 7. The handle of claim 1 in which c = 1, d=0 and Y is in the ortho position.
8. The handle of claim 1 in which c=0, d=1, and Y is in the ortho position.
- 15 9. A handle for use in peptide synthesis of the general formula:



- in which Z is [S]R<sup>4</sup> or OR<sup>4</sup>, R<sup>4</sup> is an alkyl group  
25 comprising a reactive functional group for coupling to a solid phase support; R<sup>1</sup> is an alkyl group and in which [S] is -S-, -SO-, or -SO<sub>2</sub>-; and in which D is H, a protecting group or an N<sup>α</sup>-protected acylamine.
- 30 10. The handle of claim 9 in which Z is OR<sup>4</sup>.
11. A solid phase support comprising the handle of claim 1.
- 35 12. A solid phase support comprising the handle of claim 9.

13. A method for preparing a handle for use in peptide synthesis comprising the steps of:

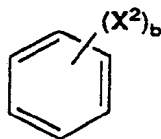
- (a) activating a benzoic acid of the  
5 formula:



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in which  $a=1$  and  $X^1$  is in the ortho or para position, or  $a=2$  and  $X^1$  is in the ortho and para position,  $X$  is  $[S]R^1$ ,  $R^1$  is an alkyl group, and  $[S]$  is  $-S-$ ,  $-SO-$ , or  $-SO_2-$ ;

- 15 (b) reacting the benzoyl chloride prepared in step (a) with a substituted benzene of the formula:



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in which  $b=0$  to 3,  $X^2$  is  $[S]R^2$ , with the proviso that if  $b > 1$ , the  $X^2$  groups are para or ortho with respect to each other,  $R^2$  is an alkyl group, and  $[S]$  is  $-S-$ ,  $-SO-$ , or  $-SO_2-$ ;

25

(c) treating the mixture of step (b) with two molar equivalents of  $AlCl_3$ ;

(d) alkylating the  $-OH$  group of the product of step (c);

30

(e) reacting the product of step (d) with hydroxylamine to yield the oxime; and

(f) reducing the oxime to yield a benzhydrylamine.

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14. The method of claim 13 further comprising the following step:

(g) reacting the benzyhydramine of step (f) with an activated FMOC at about pH 9 in aqueous solution.

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15. A method for preparing a resin for peptide synthesis comprising conjugating the handle of claim 1 or 9 to the resin.

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16. The method of claim 15 further comprising oxidizing the resin-handle conjugate.

17. A method of peptide synthesis comprising:

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(a) coupling an activated amino acid to a deprotected oxidized handle of claim 1 or 9;

(b) reducing the handle amino acid conjugate; and

(c) cleaving and deprotecting the peptide.

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18. The method of claim 17 in which a step of deprotecting the peptide preceeds the reducing step (b).

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19. The method of claim 17 in which more than one activated amino acid is coupled to the peptide prior to the reducing step (b).

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20. The method of claim 18 in which more than one activated amino acid is coupled to the peptide prior to the deprotecting step.

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# INTERNATIONAL SEARCH REPORT.

International Application No. PCT/US92/02962

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>3</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC (5): A61K 37/02; C07C 261/00 US CL : 560/27; 562/441; 525/54.11; 530/334, 341		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>4</sup>		
Classification System	Classification Symbols	
U.S.	560/27; 562/441; 525/54.11; 530/334, 341	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched <sup>5</sup>		
CAS ONLINE, APS		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <sup>14</sup>		
Category <sup>9</sup>	Citation of Document, <sup>16</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No. <sup>18</sup>
X/Y,P	Chemical Abstracts, Volume 115, No. 17, issued 28 October 1991, Patek et al., "Safety-Catch Anchoring Linkage For Synthesis Of Peptide Amides By Boc/Fmoc Strategy", see page 988, column 1, abstract no. 183861S, Tetrahedron Lett., 32(31), 3891-4.	1-12/13-20
A	Journal of Organic Chemistry, Volume 41, No. 23, issued 1976, Orlowski et al., "Study of Benzhydrylamine-Type Polymers. Synthesis And Use of P-Methoxybenzhydrylamine Resin In The Solid-Phase Preparation of Peptides", pages 3701-3705. See entire article.	13-20
Y	Journal of the Chemical Society Chemical Community, Volume 5, issued 1988, Funakoshi et al., "A Modified Benzhydrylamine As A Handle Reagent For The Solid Phase Peptide Synthesis of Peptide Amides Based On Fluorenyl Methoxy Carbonyl Method", pages 382-384. See entire article.	1-20
Y	Tetrahedron Letters, Volume 31, No. 36, issued 28 August 1990, Patek et al., "A Safety-Catch Type Of Amide Protecting Group", pages 5209-5212. See entire article.	1-20
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<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search <sup>2</sup>	Date of Mailing of this International Search Report <sup>2</sup>	
30 JUNE 1992	07 JUL 1992	
International Searching Authority <sup>1</sup>	Signature of Authorized Officer <sup>20</sup>	
ISA/US	BENNETT CELSA <i>[Signature]</i>	



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US92/02962 <b>(22) International Filing Date:</b> 10 April 1992 (10.04.92)  <b>(30) Priority data:</b> PV 1030-91                      12 April 1991 (12.04.91)                      CS  <b>(71)(72) Applicants and Inventors:</b> LEBL, Michal [CS/US]; 10700 North La Reserve Dr., #16206, Tucson, AZ 85737 (US). PATEK, Marcel [CS/US]; 10700 North La Reserve Dr., #16203, Tucson, AZ 85737 (US).  <b>(74) Agent:</b> MISROCK, S., Leslie; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).		<b>(81) Designated States:</b> AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent), US.  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> SUBSTITUTED BENZHYDRYLAMINES AS HANDLES FOR SOLID PHASE PEPTIDE SYNTHESIS  <b>(57) Abstract</b>  <p>The present invention is directed to a novel "handle" for solid phase peptide synthesis. The handles can be converted from a stable form to a labile form, allowing for cleavage of a peptide amide from the support after synthesis under mild conditions, and after deprotection of the amino acid side chains. The handles are based on a substituted benzhydrylamine skeleton.</p>		

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